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Enhancing management effectiveness of marine protected areas and coral reef species conservation through assessment of mycosporine-like amino acid (MAA) content in populations and genera

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Introduction

One of the most critical challenges to the long-term effectiveness of marine protected area (MPA) management is global climate change. Coral reefs have been shown to be extremely sensitive to both ultraviolet (UV) radiation and thermal stresses. Massive coral bleaching events have resulted from both factors, yet little is know about how to best protect coral resources from these damaging and ubiquitous stresses. This study examines the relationship between coral bleaching and protective compounds in coral (mycosporine-like amino acids) and the water column (chromophoric dissolved organic matter). We have also identified the zooxanthellae symbionts present in the coral species surveyed, in order to better understand contributing endogenous bleaching tolerance. MPA placement and management may be directed by the natural resilience generated by variation in concentrations of these protective compounds.

Coral bleaching is considered to be a stress response caused primarily by increased water temperature (Glynn 1993) and synergistically enhanced by increased irradiance levels (Jokiel and Coles 1990, Lesser et al. 1990, Fitt and Warner 1995). The El Niño-Southern Oscillation (ENSO) event of 1982-83 marked the first contemporary broad scale reef coral bleaching and mortality event (Glynn 1984). Since then there have been subsequent bleaching events including the 1997-98 ENSO event. The rate of occurrence (annually in some cases) and virtually global scale since the early 1980's is in stark contrast to the trend of the first half of the century in which bleaching events were small-scale and linked to local events (D'Elia 1991, Glynn 1993). From 1876-1979 only three bleaching events were recorded, whereas 60 are on record from 1980 until 1993 (Glynn 1993). The increase in bleaching has led to the suggestion that anthropogenic alterations of the environment are responsible, notably global climate change related increases in annual sea surface temperature and occurrence of ENSO events (Hoegh-Guldberg 1999, Pittock

1999). On the Great Barrier Reef, for example, it has been estimate that by 2050 the reef will experience thermal stress above the threshold that causes loss of reef building corals and related organisms every year. As a result coral cover may “decrease to less than 5%” on these reefs by that time (Hoegh Guldberg and Hoegh-Guldberg 2004)

Coupled with this increase in sea surface temperature (on a seasonal and episodic basis), is an increase in UV-B radiation due to both stratospheric ozone depletion and climate change. Evidence indicates that over the past two decades there has been an ozone depletion-related increase in UV-B irradiance in tropical latitudes where corals thrive (Madronich et al. 1995). This is on top of the fact that solar UV-B irradiance is naturally highest in the tropics. Additionally, although UV-B increases are generally thought of only in terms of stratospheric ozone depletion, tropical warming may also result in increased UV-B-penetrance into the water column. Studies indicate that warmer conditions, which result in decreased dissolved organic matter [including chromophoric dissolved organic matter (CDOM)] and increased stratification of the affected seas, increase UV-B penetration into the water column (Vodacek *et al.* 1997, Siegel and Michaels 1996). This means that as the stress of warmer water increases, so does the added stress of UV-B radiation. The synergistic effects of temperature and UV radiation on coral oxidative stress and bleaching are well established (Lesser *et al.* 1990, Dunne and Brown 2001)

The role of CDOM in the response of coral to climate change is subtle but potentially very important. The exposure of coral assemblages to UV radiation depends not only on the intensity of UV irradiance at the sea surface, but also on attenuation of the UV radiation as it travels into and through the water column. The penetrance of UV radiation into natural waters is highly variable (Degrandpre *et al.* 1996, Kirk 1994a, Smith and Baker 1979), ranging from meters in coastal regions to tens of meters in the open ocean. To further complicate matters, UV penetration at a given location varies with time, especially in coastal areas that are heavily impacted by runoff from land (Morris and Hargreaves 1997, Vodacek *et al.* 1997, Degrandpre *et al.* 1996).

The variability of UV penetration is attributable to changes in the composition of the water. Pure water itself is quite transparent to UV-B radiation, but UV-absorbing organic matter in most

natural waters, especially CDOM, strongly absorbs UV radiation. The attenuation of UV as it passes through ocean water can be computed from concentrations of CDOM since CDOM generally absorbs UV radiation more strongly than particulates (plankton, detritus, etc.; Vodacek *et al.* 1997, Degrandpre *et al.* 1996, Siegel and Michaels 1996). These findings suggest that CDOM may play an indirect but important role in reducing the exposure of coral assemblages to UV radiation, and this shield is directly dependent on the concentration of CDOM in overlying water. It has been suggested that such a process may occur to some extent in the Florida Keys (Anderson *et al.* 2002). CDOM, which may be acting beneficially to protect coral, is introduced to the water column by the biotic or abiotic degradation of plant matter which originates as phytoplankton, submerged aquatic vegetation or from terrestrial vegetation (Kirk 1994b). Its concentration in the overlying water is dependent on the variation and proximity of the sources, amount of terrestrial run-off, tidal and current flux and stratification, which are additionally influenced by meteorological condition and season (Conde *et al*. 2000, Gibson *et al.* 2000).

Compounds in the water are not the only potentially protective factors working in favor of healthy corals. Corals and their zooxanthellae also contain protective compounds, known as mycosporine-like amino acids (MAAs). MAAs are a suite of water soluble compounds with a central ring structure and characteristic absorption maxima ranging from 309 to 360 nm. MAAs have overlapping absorption ranges that extend across much of the spectrum of damaging UVA and UVB, creating effective broad-spectral sunscreens (Dunlap and Shick 1998). MAA concentration varies among tissues, often being highest in the tissues most exposed to UV radiation, such as ocular lenses of fishes (reviewed by Dunlap and Shick 1998), and in eggs of sea urchins and corals destined for release to the environment (Adams and Shick 1996, 2001, Michalek-Wagner and Willis 2001). MAA concentrations in corals are positively correlated with natural levels of UV radiation (Banaszak et al. 1998, reviewed by Dunlap and Shick, 1998), so that organisms may regulate concentrations of UV-absorbing compounds in proportion to the amount of UVR they experience in their environment (Dunlap and Chalker 1986, Gleason 1993, Shick *et al.* 1996, 1999). Shallow-dwelling corals and their spawned planulae have a greater resistance to UV radiation than do deep-dwelling corals (Gleason and Wellington 1995, Shick *et al.* 1996).

The synthesis of MAAs is restricted to bacteria, cyanobacteria, fungi, and algae by the occurrence of the shikimic acid pathway in these organisms (reviewed by Shick *et al*. 2000). Accumulation of MAAs by animals is attributed to translocation of MAAs from alga to host in symbiotic relationships (Shick *et al*. 1999). Although many corals alter the amount of MAAs in accordance with UV-exposure, non-symbiotic marine consumers such as sea urchins are unable to synthesize the compounds themselves and acquire MAAs from their diets of algae (Adams and Shick 1996, Carroll and Shick 1996). MAAs have been shown to have a protective role in marine invertebrates and phytoplankton. MAAs protect against UV-induced reductions in primary productivity in phytoplankton (Neale *et al.* 1998). Accumulation of MAAs in the eggs of sea urchins protects them in part from UV-induced delays in cleavage (Adams and Shick 1996) and abnormalities during later development (Adams and Shick 2001). Fortification of eggs with MAAs may decrease the chances of cellular damage and developmental abnormalities.

The belief exists that MAAs play a role in protecting corals from bleaching, however the research to support that contention has not been developed. MAAs have been shown to protect against UV-induced reductions in photosynthesis in microalgae (Neale *et al*. 1998). Similarly, a precursor to bleaching is decreased photosynthesis of symbiotic zooxanthellae. It has been demonstrated that UV-exposure decreases photosynthesis in cultured zooxanthellae (Lesser 1996), but not in colonies of coral with this zooxanthellae when the host tissues contained high concentrations of MAAs (Shick *et al.* 1996), indicating that MAAs may protect against some of the initial manifestations of coral bleaching if and when they are caused by synergistic effects of temperature and UV radiation.

Eventually, concentrations of MAAs in adult coral tissues may be reduced by bleaching (Michalek-Wagner and Willis 2001). Typically, most MAAs are quite stable and not susceptible to complete degradation, so it is unclear whether these MAAs are leached out of tissues or whether they are degraded during a photoprotective process. Therefore, it appears likely that they may be released into the water column in combination with CDOM, a process that occurs in microalgal blooms (Vernet 2000). The combined presence of CDOM and MAAs in the water column may provide additional protection for corals against UV-irradiation, but this hypothesis has not been examined to date.

Another endogenous factor that may increase the resistance or resilience of coral to bleaching is the zooxanthellae symbiont. It is now clear that the genus *Symbiodinium* is exceptionally diverse and consists of up to eight major clades (A-H, Pawlowski *et al.* 2001, Pochon *et al.* 2001, Baker 2003). Members of five of these clades (A, B, C, D and F) have to date been documented in scleractinian corals. Many scleractinian corals are relatively flexible in the types of symbionts they can contain (Rowan and Knowlton 1995, Rowan *et al.* 1997, Baker 1999, Baker 2001, Baker 2003) although one type is usually dominant in any given species and environment (Rowan & Powers 1991, LaJeunesse 2002, LaJeunesse *et al.* 2003). Consequently, because the phenotype of these associations is an emergent property of two (or more) interacting genotypes – host and symbiont(s) – the response of reef coral symbioses to environmental conditions cannot be fully understood without an explicit understanding of the identities of all partners.

Reef corals are known to associate with diverse algal symbionts, and in many cases are able to switch or shuffle these symbionts in response to environmental change (Rowan *et al.* 1997, Rowan 1998, Baker 2001, Baker 2003, Baker *et al.* 2004, Little *et al.* 2004). Certain types of symbiont in particular (symbionts in *Symbiodinium* clade D and C15), appear to be unusually resistant to high temperature stress.

Materials and Methods

Field Collection

Coral was collected from four locations around the American Samoa island of Tutuila in the South Pacific Ocean (approx. 170°W and 14°S). Collection occurred in March, June and August 2003 and February 2004. Tutuila locations were Fagatele Bay National Marine Sanctuary and Faga'itua Bay on the south shore, and Vatia and Tafeu Bays (both within the National Park of American Samoa) on the north shore (Figure 1).

Figure 1. Field collection sites on Tutuila Island, American Samoa. Coral and water samples were collected from Fagatele Bay NMS, Faga'itua Bay, Vatia Bay, and Tafeu Cove. Water samples were additionally collected from Leone Bay, Faga'alu Bay, and Malota Bay.

At the Tutuila sites five nubbins (each two to three centimeters in length) were collected with bone cutters from the tips of branching corals from one colony of each of the following species (when present): *Acropora formosa*, *Pocillopora eydouxi* and *Porites rus*.

Samples were placed into sealed plastic bags with seawater and transported back to the boat or beach in a black-sided game bag. Onshore, coral were transferred to a cooler filled with ambient temperature seawater for temporary storage. Corals were blotted dry and transferred to individual Whirl-pak bags (Nasco, Modesto, CA), chilled to -20°C and then placed into a liquid nitrogen-chilled shipping dewar. Coral were kept out of direct sunlight during all handling.

Fragments were chipped off for zooxanthellae identification either in the field prior to freezing or after arrival at the laboratory. Field prepared samples for zooxanthellae identification were placed in saline, pH 8, 20% dimethylsulfoxide (DMSO) buffer and shipped at room temperature to Dr. Andrew Baker for analysis. The remaining samples were shipped frozen, without buffer, following processing at California Polytechnic State University by Dr. Nikki Adams.

Water samples for CDOM analysis were collected over a larger date range and at more sites than the coral. These dates were October 2002, March, June, August 2003, February, and July 2004.

The added sites included Leone Bay, Faga'alu Bay, and Malota Bay on Tutuila Island. Aliquots for analysis were prepared from bulk grab samples collected during coral sampling and survey work or obtained from the major stream of the watershed above each reef location. Stream samples were collected at outflows just above tidal influx. All CDOM water samples were filtered (0.2 µm; stream samples were 0.7 µm pre-filtered) and stored refrigerated in foil-wrapped polycarbonate bottles until analysis. Samples were shipped to Dr. Richard Zepp at the U.S. Environmental Protection Agency, Athens, Georgia for analysis.

Table 1 provides a complete overview of sites and samplings. Sample collection was occasionally hampered by weather and logistical difficulties resulting in missing samples at Fagatele Bay, Faga'alu Bay, Tafeu Cove and Malota Bay.

Extraction and analysis of mycosporine-like amino acids (MAAs)

Coral samples were shipped to California Polytechnic State University, San Luis Obispo frozen on dry ice. Samples were then stored at –80°C until analysis for MAAs. The concentration of MAAs in coral tissues was determined using high performance liquid chromatography (HPLC). Frozen corals were pulverized and weighed (~2-4g wet weight), and MAAs were extracted by three or four serial 60-min extractions in 100% HPLC grade aqueous methanol at room temperature in the dark. MAAs were separated by reverse-phase HPLC, identified, and quantified by examining the absorption maximum of each peak and using quantitative standards prepared by Dr. W. C. Dunlap (Australian Institute of Marine Science, Townsville). Peak separation was achieved in a mobile phase composed of 55% aqueous methanol and 0.1% acetic acid using a Hewlett Packard 1100 HPLC and a flow rate of 0.8 ml/min, with diode array detector. Concentrations of MAAs are presented based on protein concentration. Extraction efficiencies for individual MAAs in each species of coral were calculated (Adams and Shick 1996), and used to correct the concentration of MAAs.

Determination of Protein Concentration

Proteins were extracted from coral tissue using 0.1N NaOH at 90°C for 30 mins. Extracts were cooled to room temperature and neutralized using HCl. Protein concentrations of extracts were determined using the Pierce BCA protein assay.

CDOM analysis

Optical measurements of CDOM were performed with an Agilent Model 8453 Diode Array Spectrophotometer equipped with 1 cm or 10 cm quartz cells. Absorption spectra in the UV-visible region from 220 to 1100 nm were measured and baseline corrected by subtraction of the average absorbance in the 700 to 725 nm range (Blough & Del Vecchio 2002). Absorption coefficients were calculated with the following equation:

$$a_\lambda = 2.303x(A_\lambda / L)$$

where A_λ is the absorbance at a specific wavelength and L is the cell pathlength. A broad and featureless exponential decrease in absorbance coefficient with increasing wavelength is typical of CDOM. Absorption coefficients at 305 nm and 310 nm are considered most indicative of UV-B irradiance at depth and is reported here.

The spectral slope coefficient (S) is a parameter that is related to the 'nature' or source of CDOM and quantifies the relationship between the absorption coefficient and wavelength. Spectral slope coefficients were calculated for the wavelength range of 290 to 500 nm or 290 to 400nm using the following non-linear fit equation (Blough and Del Vecchio 2002):

$$a_\lambda = a_{\lambda_0} e^{S(\lambda_0 - \lambda)}$$

where a_λ is the absorption coefficient at wavelength λ, $a_{\lambda 0}$ is the absorption coefficient at a reference wavelength λ_0, and S is the spectral slope coefficient.

Zooxanthellae Identification

Identification of symbiont taxa used established molecular techniques involving Restriction Fragment Length Polymorphisms (RFLPs) in large subunit (28S-like) rRNA genes (Rowan and Powers 1991, Baker *et al.* 1997, Baker and Rowan 1997, Baker 1999). The conserved primers 24D15F1 and 24D23R1 were used and symbionts were initially identified to the level of clade.

Denaturing Gradient Gel Electrophoresis (DGGE) was then used to separate PCR-amplified products of the Internal Transcribed Spacer-2 (ITS-2) ribosomal DNA of *Symbiodinium* (per LaJeunesse 2001, LaJeunesse 2002) and distinguish mixed symbiont communities. Novel symbiont types were sequenced and compared to a Genbank-archived database that currently comprises >100 distinct *Symbiodinium* taxa. These methods give results of high taxonomic resolution (see Baker 2003 for review) and are popular for identifying the algal symbionts within scleractinian corals (but see Santos *et al.* 2003, Santos *et al.* 2002a, Santos *et al.* 2002b for alternative approaches successfully applied to gorgonian corals).

These methods fail to distinguish taxa that represent less than 5-10% of the symbiont population. It should therefore be kept in mind that additional minor symbiont types may be present in this samples below the threshold level for molecular resolution using the techniques described above.

Statistical Analysis

MAA data were analyzed using split plot analysis of variance ANOVA (split plot by date) and Student-Newman-Keuls (SNK), using StatView 5.0.1 (SAS Institute Inc.). CDOM data were tested for normal distribution using the Shapiro-Wilk test, log transformed [$\log_{10}(x+1)$] if necessary, and compared by ANOVA or *t* test using SYSTAT 11 (Systat Software, Inc.). Data were combined either by date or by location for interpretation. Data are reported as means ± one standard deviation where appropriate.

Results

Mycosporine-like amino acids (MAAs)

There was a significant difference in the concentration of total MAAs in *A. formosa* among sites ($P=0.004$) and dates ($P=0.004$) and there was a significant interaction among dates and sites ($P=0.0024$). Data for all Tafeu samples and June 2003 were not included in this analysis because not all sites were sampled. There was a significant difference in the concentration of total MAAs between March 2003 and both August 2003 and February 2004, but not between August 2003 and February 2004. There was a significant difference in the concentration of total MAAs over time at Fagatele ($P=0.043$). MAA concentrations on August 2003 were significantly higher than February 2004 ($P<0.05$), but there was no difference in MAA concentrations between March

2003 and August 2003 or March 2003 and February 2004 (P>0.05). There was a significant difference in total MAA concentration over time at Vatia (P=0.0001) and Faga'itua (P=0.0145). MAA concentrations at Vatia were greater in March 2003 than all other dates, which were not significantly different from each other (P< 0.05). Total MAA concentrations at Faga'itua were greater in March 2003 than June 2003 and February 2004 (P<0.05), but there were no other significant differences in MAA concentrations among dates. (Figure 2)

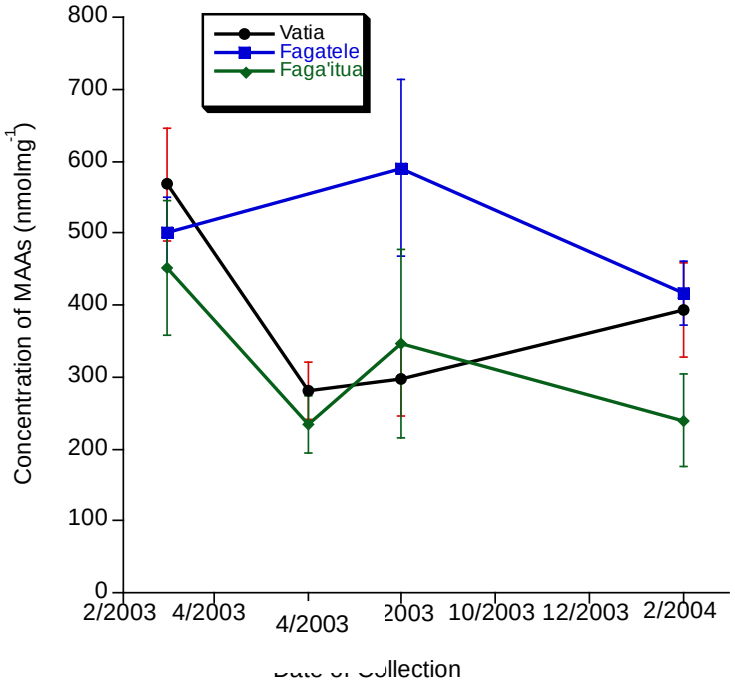

Figure 2. Concentration of mycosporine-like amino acids (± SD, n=4) in *Acropora formosa* among collection sites over time.

Data were also analyzed for differences in total MAA concentration by date. In March 2003 there was no significant difference in MAA concentrations among sites (P=0.148). In August 2003 there was a significant effect of site (P=0.0084). Total MAA concentration was significantly higher at Fagatele than both Faga'itua and Vatia (P<0.05). In February 2004, there was no significant differences among sites (P=0.15).

There was a significant difference in the concentration of total MAAs in *P. eydoux* among sites (P=0.033) and dates (P=0.003), but there was no significant interaction among dates and sites

(P=0.127) (Figure 3). Data for all June 2003 were not included in this analysis because not all sites were sampled. These data were examined in separate analyses by site.

The concentration of total MAAs in March 2003 and February 2004 were both significantly higher than in August 2003 (SNK, P<0.05), but there was no difference in MAA concentrations between March 2003 and February 2004 (SNK, P>0.05). Total MAA concentrations were significantly higher at Vatia that Faga'itua (SNK, P<0.05), but there were no other differences among sites. There was no difference among the total concentration of MAAs among time points at Vatia (P=0.0519) or Faga'itua (P=0.7113) over time.

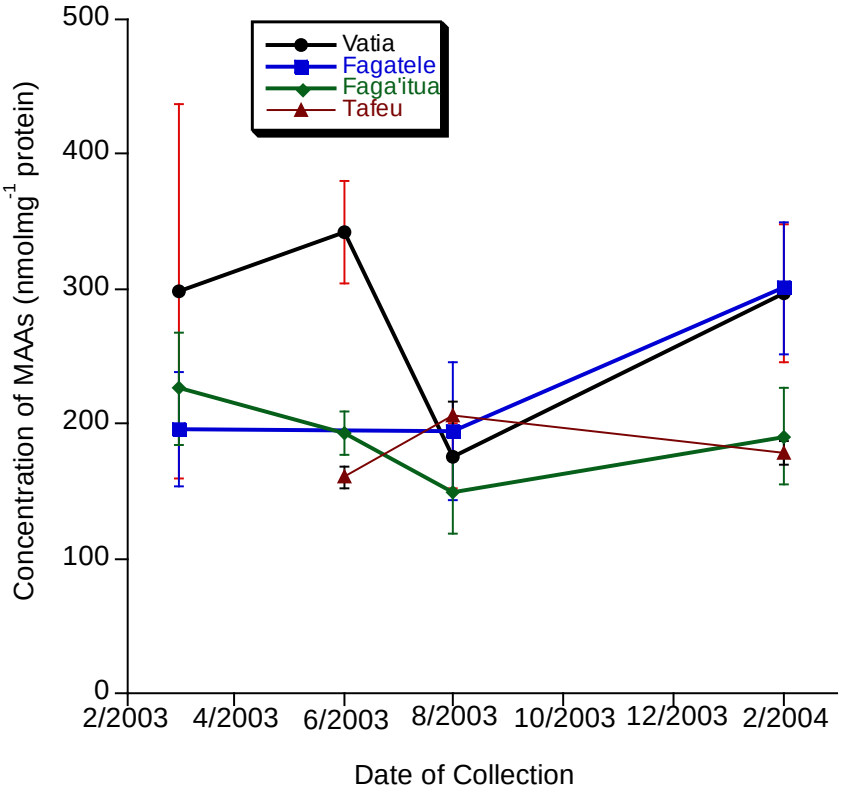

Figure 3. Concentration of mycosporine-like amino acids (± SD, n=4) in *Pocillopora eydoux* among collection sites over time.

There was a significant difference in the concentration of total MAAs in *P. rus* among sites (P=0.0001) and dates (P=0.0001) and that there was a significant interaction among dates and

sites (P=0.0018). All samples for Fagetele and those for March 2003 were not included in this analysis because not all sites were sampled. (Figure 4)

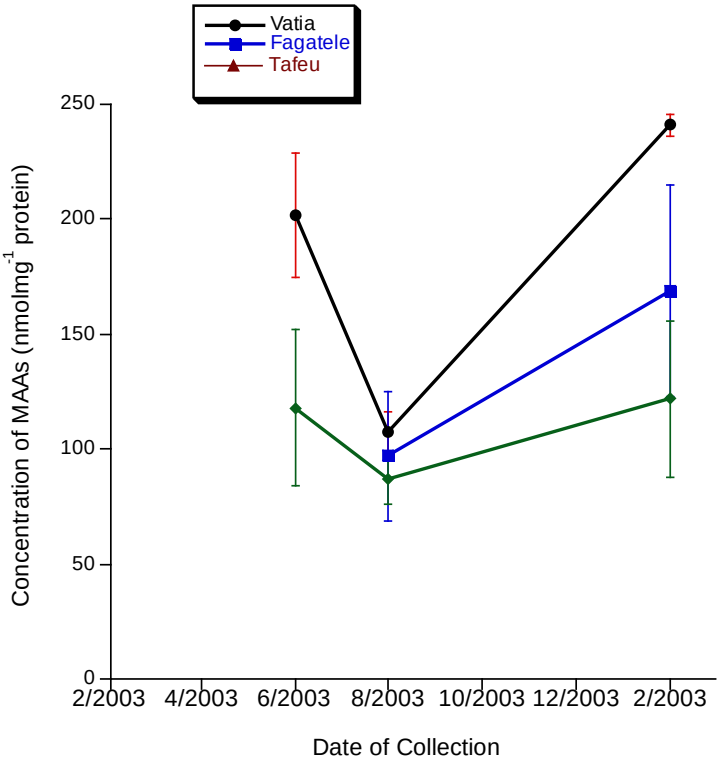

Figure 4. Concentration of mycosporine-like amino acids (± SD, n=4) in *Porites rus* among collection sites over time.

There was no significant difference in total MAA concentration among dates at Tafeu (P=0.210). There was a significant difference in total MAA concentration among dates at Vatia (P=0.0001). The total MAA concentration in *Porites* at this site was significantly greater in February 2004 than in both June 2003 and August 2003. MAA concentrations were significantly higher in June 2003 that August 2003.

Data were also analyzed for differences in total MAA concentration by date. There was a significantly higher concentration of total MAAs at Vatia then Tafeu in June 2003. There was no significant difference in total MAAs concentrations among three sites (Vatia, Tafeu and Fagetele) in August 2003. There was a significant difference in total MAA concentrations among sites in February 2004 (P=0.022). Total MAA concentrations were significantly higher at

Vatia than both Tafeu and Fagetele ($P<0.05$), but there was no difference in MAA concentration of coral tissues between Tafeu and Fagetele.

Complete MAA results can be found on Table 2.

CDOM

CDOM in water samples was characterized by calculating absorption coefficients (*a*) at two wavelengths within the UV-B range, and by determining the spectral slope coefficient (*S*) of the absorption curve between 290 and 500nm. CDOM in reef and stream water differed fundamentally. The mean absorption coefficients for all reef samples were 0.218 $m^{-1} \pm 0.048$ (305nm) and 0.196 $m^{-1} \pm 0.045$ (310nm). Absorption coefficients for stream samples were significantly greater at 6.830 $m^{-1} \pm 6.909$ (305nm) and 6.391 $m^{-1} \pm 6.500$ (310nm) ($p \leq 0.0001$). Comparison of spectral slope coefficients for reef and stream water showed a significant separation around 0.015 nm^{-1} , with reef water averaging 0.0179 $nm^{-1} \pm 0.0029$ and stream water averaging 0.0146 $nm^{-1} \pm 0.0007$ ($p \leq 0.0001$).

Absorption coefficients and spectral slope coefficients differed significantly for reef water over time (Figure 6 and Figure 8), however there were no differences when locations were compared (Figure 5 and Figure 7). Reef water showed a trend of increasing absorbance over time at both wavelengths (Figure 6), and the mean absorbance coefficient for the last reef water sample of the study (July 2004) was statistically different than 2002 and 2003 samples ($p \leq 0.015$). Spectral slope coefficients for reef water were consistent except for February 2004 which was significantly different than previous sample periods ($p \leq 0.033$) (Figure 8).

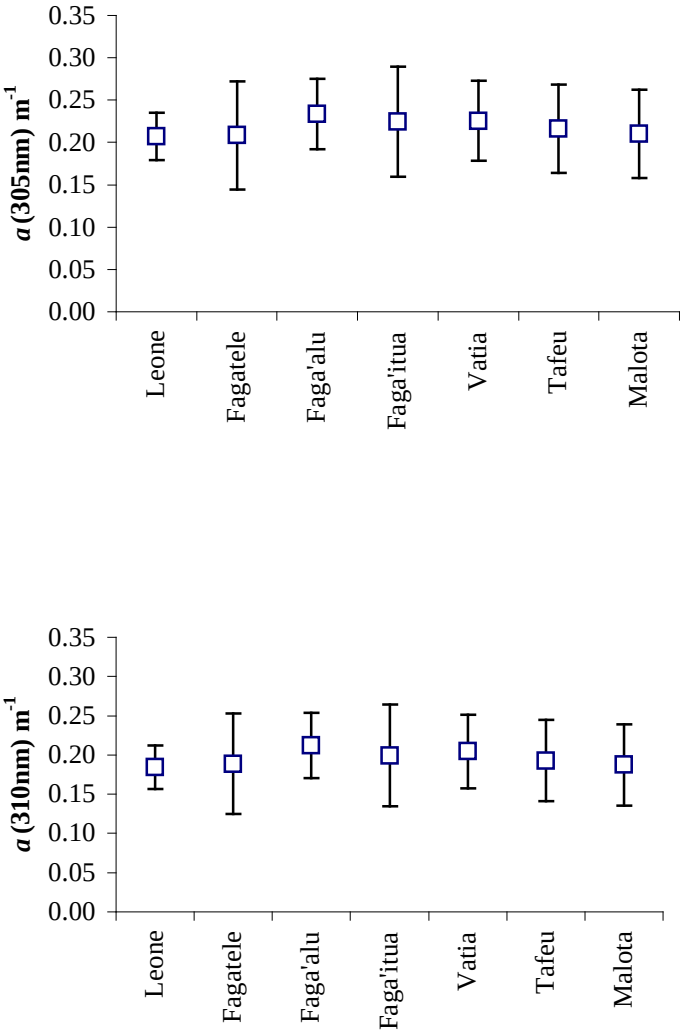

Figure 5. Reef water CDOM influenced absorption coefficients for seven sites around Tutuila Island. There are no statistical differences between sites for 305 or 310nm. (mean ± SD, n ≥ 4)

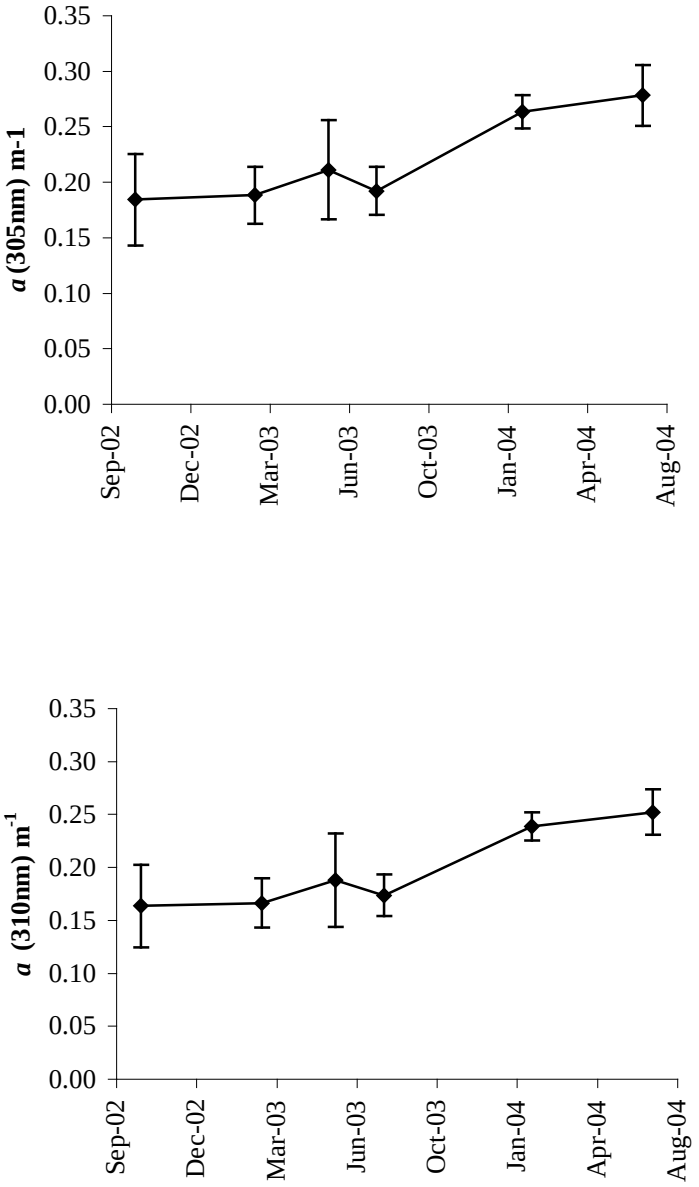

Figure 6. Reef water CDOM influenced absorption coefficients over time on Tutuila Island. July 2004 time-point significantly greater than previous dates for both wavelengths ($p \leq 0.015$). (mean ± SD, $n \geq 5$)

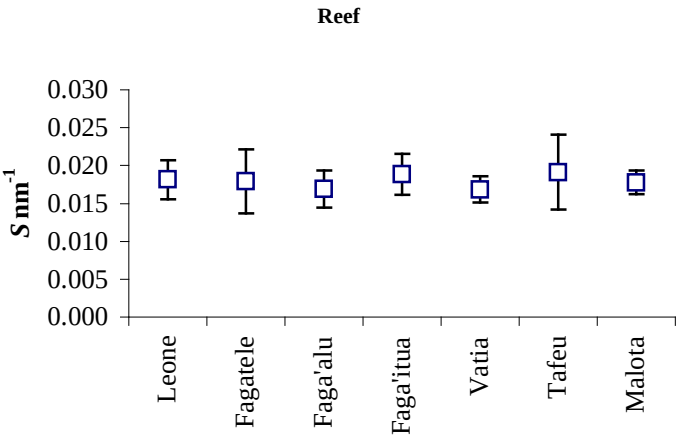

Figure 7. Calculated spectral slope coefficients for seven reef sites around Tutuila Island. There were no statistical differences between sites (mean ± SD, $n \geq 4$).

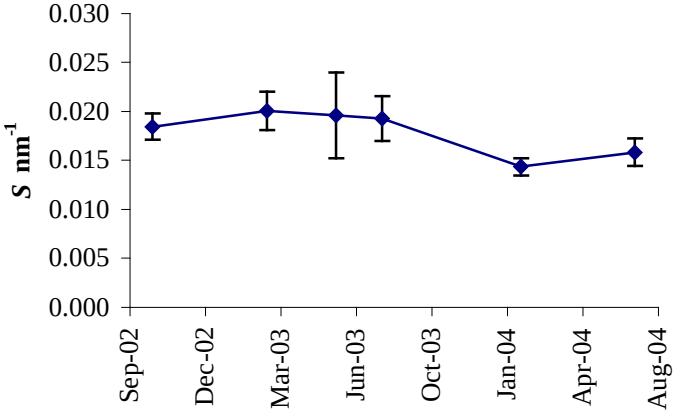

Figure 8. Spectral slope coefficients for combined reef sites over time. February 2004 samples were on average less than previous time-points ($p \leq 0.033$). (mean ± SD, $n \geq 5$)

Analysis of stream water samples revealed differences between locations for mean absorption coefficients at both wavelengths and one spectral slope coefficient outlier. The mean absorption coefficients for Faga'itua were greater than all sites except Vatia ($p \leq 0.027$) at both 305 and 310nm when all sample periods were combined (Figure 9). The mean absorption coefficient for Vatia was also elevated, but only relative to Fagatele and Malota ($p \leq 0.050$). Spectral slope

coefficients for all stream bottom sites were similar except for the Fagatele stream ($p \leq 0.0001$) (Figure 10). This difference is likely due to the source which is a nearby spring. Variability between sites when combined at each time-point resulted in large standard deviations around mean absorption coefficients for stream samples (Figure 11). Thus there were no trends in absorbance observed over time. Mean spectral slope coefficients were constant over time for stream samples (Figure 12).

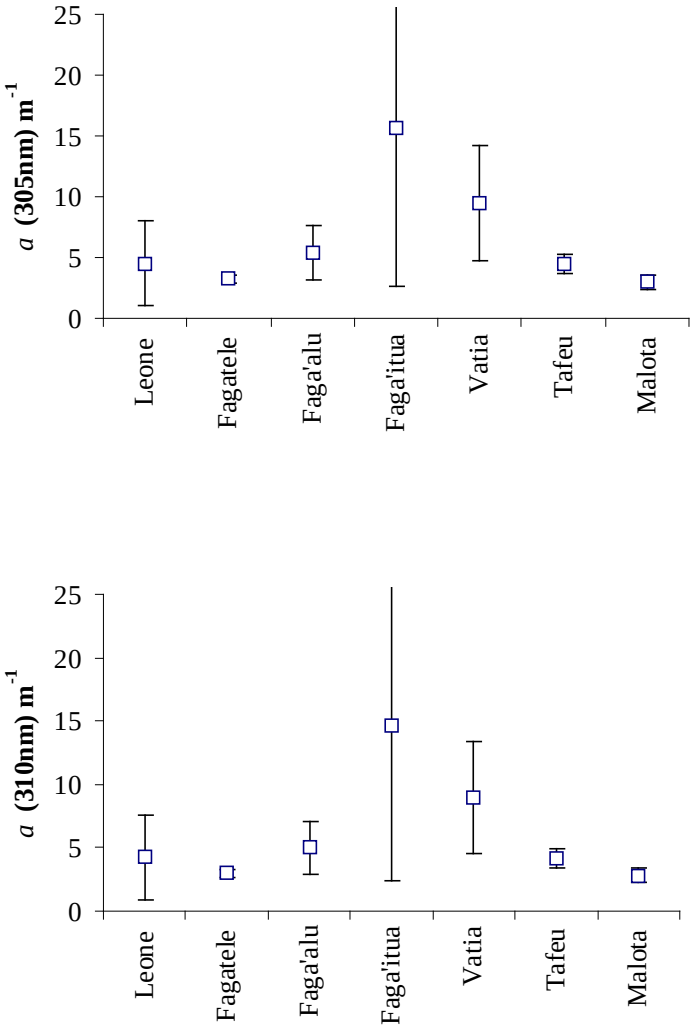

Figure 9. CDOM influenced absorption coefficients for the outflow of streams above the reef sites Faga'itua was greater than all sites except Vatia ($p \leq 0.027$). (mean ± SD, $n \geq 4$)

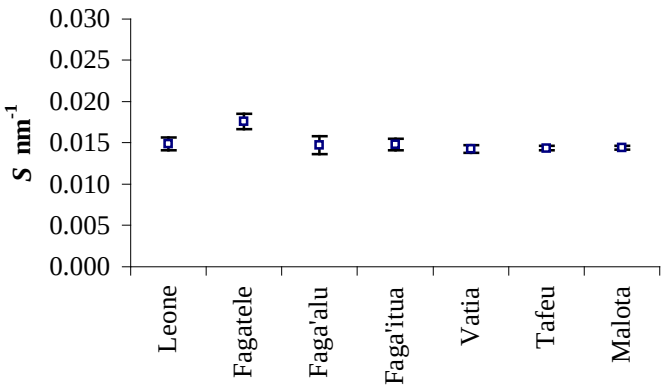

Figure 10. Spectral slope coefficients for stream outflows above reef sites. Fagatele is unique among the sites ($p \leq 0.0001$). See discussion. (mean ± SD, $n \geq 4$)

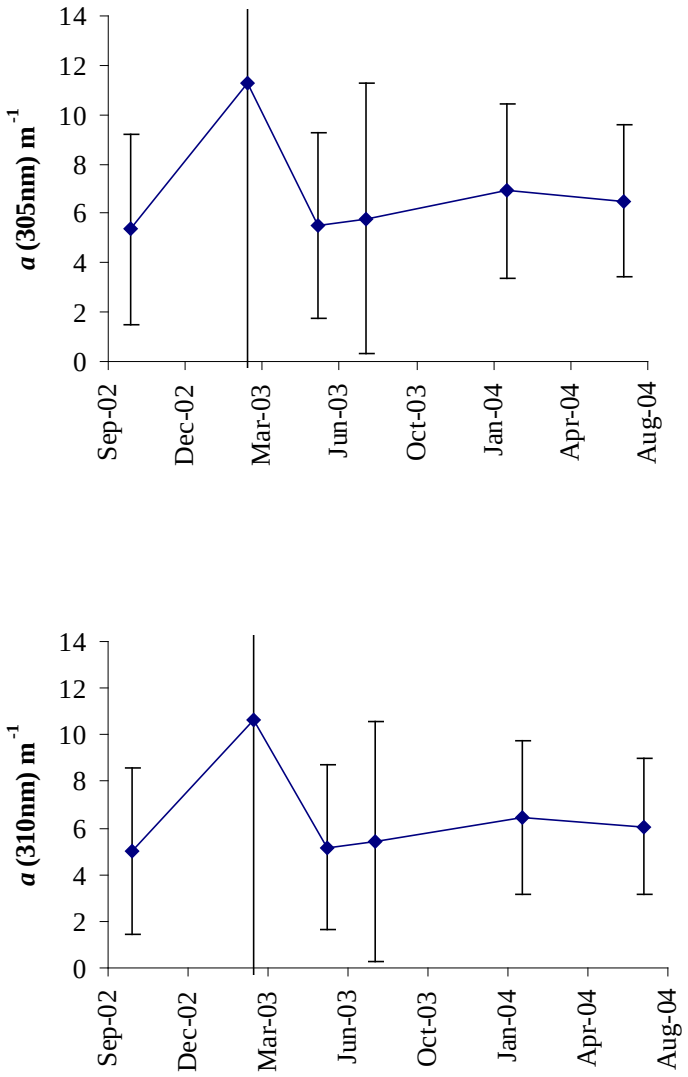

Figure 11. CDOM influenced absorption coefficients for combined stream outflows over time. No statistical difference between sample periods was found. (mean ± SC, n ≥ 5)

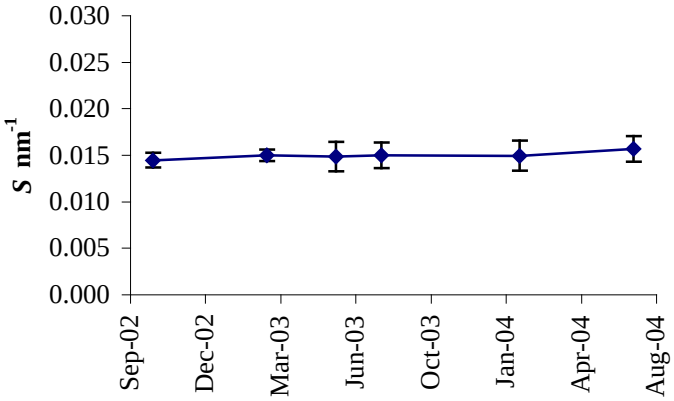

Figure 12. Spectral slope coefficients for combined stream outflow over time. There were no differences between sample periods. (mean ± SD, n ≥ 5)

Zooxanthellae

Zooxanthellae identification was conducted on both chemically-preserved and frozen samples. There appears to be no variation due to this difference in sample treatment. However there do seem to be strict patterns regarding genera and symbionts. For the Acroporids, 8 of the 11 samples contained only *Symbiodinium* D2. Samples from August 2003 from Fagatele and Vatia contained only *Symbiodinium* C1c. The February 2004 sample from Faga'itua contained only *Symbiodinium* A3.

In the Genus *Pocillopora*, 10 of 12 samples contained only *Symbiodinium* C1c. The remaining 2 samples, from Vatia in March and June 2003, contained only *Symbiodinium* C3. In the Genus *Porites*, all 8 samples contained only *Symbiodinium* C15. (See Table 3).

Discussion

The results from American Samoa indicate that *Acropora*, from the locations sampled, has a propensity to host *Symbiodinium* in clade D, which recent findings indicate may be unusually tolerant to heat stress (Baker 2004, Baker *et al.* 2004a, Baker *et al.* 2004b). However, a few samples also contained A3 and C1c, indicating that (A3) these corals may also be considered representative of relatively high latitudes (Baker 2003) and that (C1c) this genus can

occasionally mix symbiont types within single colonies. Of the three genera of coral investigated in this study, *Acropora* appears to be the most flexible in its associations.

The genus *Porites* in American Samoa appears to be very specific in its associations, with all samples analyzed containing only *Symbiodinium* C15. This is a general rule for Pacific *Porites*, and these results are not surprising. Some evidence exists that this is also a high temperature-tolerant symbiont type, suggesting that these colonies may also be relatively resistant to bleaching.

The genus *Pocillopora* is also somewhat flexible in its associations, although most samples contain only *Symbiodinium* C1c. Two samples contained *Symbiodinium* C3, which is relatively closely-related to C1c. Due to the absence of clades D or C15, it would be predicted that the Pocilloporids would in general be more susceptible to bleaching than the other two genera at these locations.

The MAA concentrations that we report are fairly consistent with concentrations of MAAs reported for these genera in other locations, although perhaps on the high end in the case of the Acroporids. The *A. formosa* from American Samoa had MAA concentrations between 230 and 641 nmol mg^{-1} protein. *A. microphthalma* on the Great Barrier Reef in Australia have been reported to have total MAA concentrations ranging from 30-250 nmol mg^{-1} protein and which correlate negatively with depth (i.e. shallow swelling corals have the highest concentrations of MAAs) (Shick *et al.* 1995).

Porites porites from samples collected at Carrie Bow Cay, Belize ranged from 100-600 nmol mg^{-1} protein and correlated negatively with depth. Two other species of *Porites* from Hawaiian reefs *(P. compressa, P. lobata (purple), and P. lobata)* had the following average total MAA concentrations respectively 133, 71 and 22 nmol mg^{-1} protein respectively (Banaszak *et al.*1998). *Porites rus* from American Samoa contained between 85 and 241 nmol mg^{-1} protein, perhaps high for the Pacific but not globally.

Hawaiian *P. damicornis* tissue concentrations of total MAAs have ranged from 4 to >2,000 nmol mg^{-1} protein pursuant to manipulations of UV and water flow regimes (Jokiel *et al.* 1997). Two additional species of *Pocillopora* from Hawaiian reefs *(P. eydouxi, and P. meandrina)* had average total MAA concentrations of 30 and 32 nmol mg^{-1} protein respectively (Banaszak *et al.* 1998). American Samoan *P. eydoux* had between 148 and 342 nmol mg^{-1} protein. Compared to their Hawaiian counterparts, *P. eydoux* in American Samoa is significantly higher. This might be due to the average higher UV irradiance and temperatures experienced in American Samoa.

In general the MAA concentrations that we have measured in these three species of coral follow the seasonal patterns previously reported. Previous studies of soft corals show seasonal trends. MAA concentrations in southern hemisphere populations peaked in February and were lowest in June (Michalek-Wagner 2001). In all three of our species, when there was a statistical difference between times, the February/March sampling points contained the highest concentrations of MAAs, with the exception of Fagatele Bay NMS Acroporids. At this site, August had the highest MAA concentrations. However we also saw a change in zooxanthellae population at this sampling point in two of our locations, Vatia and Fagatele. It is interesting to note at Vatia and Fagatele had the highest bleaching frequencies in the February 2003 bleaching event of the sites from which coral was collected in this study. Whereas all of the other sampling times at these sites contained D2, a more heat stress tolerant clade, the August 2003 sampling event contained C1c, the same clade that was found in the *Pocillopora*. It is interesting that this clade, which is less tolerant of heat stress, occurred in an *Acropora* in August, a time when heat stress is lowest.

CDOM

The results obtained in this study showed no significant variation in CDOM influenced UV-B light absorption between the seven reef sites. The absorbance coefficients measured at 305 and 310 nm for the reef sites were consistent and were unusually low for coastal locations. These low values indicate that little absorbance of UV-B radiation is occurring in the water column at these sites. The CDOM in the reef waters was also primarily from the open ocean, based on calculated spectral slope coefficients. This suggests that terrestrial and nearby coastal sources of CDOM were insignificant at these sites during these quarterly sampling events.

Studies have shown that the magnitude of CDOM absorbance varies greatly across marine, coastal, and riverine waters with CDOM absorption coefficient values ranging from >30 m^{-1} for coastal and riverine waters to < 0.5 m^{-1} (305nm) for oligotrophic open ocean surface waters (absorbance coefficients recalculated[1] from a review by Blough and Del Vecchio 2002). Spectral slope coefficients vary with the source of CDOM and extremes can range from approximately 0.01 nm^{-1} for terrestrial systems to as high as 0.02 – 0.03 nm^{-1} for CDOM in oligotrophic open ocean water (Blough and Del Vecchio 2002).

CDOM composition and its effects on light attenuation in the water column have been well studied around the Florida Keys and in the nearby Gulf of Mexico and Straits of Florida (Zepp 2003, Blough and Del Vecchio 2002, Green and Blough 1994, Stabenau *et al.* 2004, Coble *et al.* 2004). The Florida Keys are an area where CDOM influenced light attenuation varies geographically and over short (tide-cycle) and long (seasonal) timescales (Zepp 2003). This variability has been attributed to strong terrestrial influences from South Florida, a shallow ocean shelf, and extensive mangroves and sea-grass beds in some areas (Zepp 2003, Stabenau *et al.* 2004). In this region absorbance coefficients <0.5 m^{-1} (305nm) and spectral slope coefficients >0.02 nm^{-1} occur primarily in offshore ocean samples. River outflows and estuaries report absorbance coefficients >5 m^{-1} (305nm) and spectral slope coefficients <0.015 nm^{-1} (Green and Blough 1994, Blough and Del Vecchio 2002; absorbance coefficients recalculated[1]).

Near-shore water composition is often influenced by terrestrial and coastal inputs of CDOM resulting in greater UV light attenuation with associated unique spectral slope characteristics. The influence of nearby terrestrial inputs on CDOM at the reef sites in American Samoa was explored by quantifying the optical characteristics of nearby stream outflows to see if those characteristics were apparent in water above nearby reefs. CDOM influenced UV-B absorption coefficients in American Samoa stream water varied by location and were approximately 15 to 70 times greater than nearby reef water. However, there was no correlation in stream outflow and reef water composition. This was further reinforced by a clear separation in spectral slope coefficients for stream and reef water (Figure 7 & 10). Spectral slope coefficients in stream water were generally <0.015 nm^{-1} and >0.018 nm^{-1} for reef water. The optical characteristics of

[1] a (305) has been calculated using $a_{305} = a_{\lambda} e^{-S(305-\lambda)}$ using the reported S value.

Fagatele stream water were unique in this study. Unlike all other streams that had surface flows throughout their watersheds, the source of the Fagatele stream is an underground spring that emanates from the cliff face near the shoreline.

The CDOM influenced optical characteristics of the water above the fringing coral reefs in American Samoa are quite different from the well studied Florida Keys. This is likely due to major differences in oceanography and geology between the two. Tutuila Island is relatively small with no major rivers and is distant from any other major land mass. Terrestrial sources of CDOM by stream input to reef areas was determined to be negligible at the seven study sites and there are no known sea-grass beds or noteworthy mangrove areas. Even though the reef sites under investigation were quite near-shore and proximate to stream outflow, open ocean water dominates the reef areas. Ocean depths can exceed 200m within 0.5 nautical miles offshore and continue to quickly drop to over 1000m. Upwelling and mixing with offshore water apparently dominates coastal waters.

Conclusion

This project had three defined objectives:

1. Determine if MAA concentrations play a role in the prevention of bleaching;
2. Determine if there is a link between MAA concentrations and CDOM concentrations;
3. Use these relationships to improve MPA management.

Objective 1:

Elevated MAA concentrations during the high UV irradiance, high temperature time of the year may be an adaptive measure to protect against bleaching. Another interesting pattern was the overall MAA concentrations between the genera. Speaking in broad terms, the *Porites* had the lowest MAA concentrations (not corrected for zooxanthellae number) and the *Acropora* the highest (again, not corrected for zooxanthellae number). The zooxanthellae composition indicates that *Pocillopora* should be most likely to bleach as it contains the least tolerant *Symbiodinium* clade. In fact initial analysis from a concurrent study indicates that at during this

study period *Pocillopora* does have the highest bleaching frequency, followed by *Acropora*. An intriguing but untested hypothesis might be that clade C15 conveys its protective advantage, or tolerance, through a mechanism other than MAAs, whereas D2, the clade in the *Acropora* is in fact a good MAA producer. However with no consistent pattern of elevated MAAs in a particular site it is not yet possible to determine if MAA concentrations are a good indicator of degree of protection.

Objective 2:

The overall reef homogeneity of very low CDOM concentrations makes it impossible to determine if there is a relationship between CDOM and MAAs. The CDOM concentrations in the water over the reefs in American Samoa are much lower than in other regions where it has been measured, such as the Florida Keys. As a result this relationship would be better explored in the Florida Keys where there are higher concentrations and greater variability in the spatial and temporal distribution of CDOM.

Objective 3:

Despite the attractive nature of American Samoa being a fairly well contained study area it seems that endogenous factors such as MAAs, CDOM and zooxanthellae are not the driving factors in bleaching variability. Rather it may be other environmental factors that are responsible for variability in bleaching and recovery. Many of these factors are also being explored in two companion studies; one examines these same variables in an extremely bleaching resistant shallow lagoon on Ofu Island in American Samoa (Mielbrecht and Hansen in preparation), while the other examines the same sites as this study but also quantifies bleaching, nutrient run-off from terrestrial sources and temperature variability (Hansen and DiDonato 2002). The data from all three of these studies will be integrated following completion of the remaining projects.

There is also speculation that the lack of heterogeneity in any of the endpoints is due to selective pressure in response to regional environmental stresses resulting in them having already been optimized to the extent possible. The generally high concentrations of MAAs and the uniformity

of the zooxanthellae may support this idea. Additionally the species with the most susceptible zooxanthellae clade, *P. eydoux* had MAA concentrations that may be on the higher end of what that species may contain, although small sample sizes limit this interpretation.

Reflecting on the results of this study it seems that sampling frequencies may have been inadequate to identify variability in all of the factors examined. This may have been most significantly limiting with the CDOM data.

Despite the limitations of this information in American Samoa, it is still vitally important to consider the current and future implications and manifestations of climate change in MPA design and management. Failure to do so will hinder the success of this valuable conservation tool.

For American Samoa, it seems that these endogenous factors will not prove useful in making management recommendations. Our recommendation for future studies along this line would be to increase sampling frequency (weekly perhaps with an eye toward storm events) and conduct additional studies in regions with greater inherent CDOM variability.

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Literature Cited

Adams, N.L. and J.M. Shick.1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin, *Strongylocentrotus droebachiensis*. Photochemistry and Photobiology 64:149-58.

Adams, N.L. and J.M. Shick, 2001. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. Marine Biology 138:267-280.

Anderson, S., R. Zepp, J. Machula, D. Santavy, L. Hansen, G. Cherr and E. Mueller. 2002. Indicators of UV exposure in corals and their relevance to global climate change and coral bleaching. Human and Ecological Risk Assessment 7(5):1271-1282.

Baker, A.C. 1999. The symbiosis ecology of reef-building corals. Ph.D. thesis, Coral Gables, FL.

Baker, A.C. 2001. Reef corals bleach to survive change. Nature 411:765-766.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: Diversity, ecology and biogeography of *Symbiodinium*. Annual Review in Ecology, Evolution and Systematics 34: 661-689.

Baker, A.C. 2004a. Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. *In* Rosenberg, E. and Y. Loya (eds). Coral Health and Disease. Springer-Verlag, Berlin. pp 177-194.

Baker, A.C., C.J. Starger, T.R. McClanahan , and P.W. Glynn. 2004b. Corals' adaptive response to climate change. Nature 430: 741.

Baker, A. C. & Rowan, R. 1997. Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals of the Caribbean and Eastern Pacific. Proceedings of the 8th International Coral Reef Symposium 2:1301-1306.

Baker, A. C.; Rowan, R. & Knowlton, N. 1997. Symbiosis ecology of two Caribbean acroporid corals. Proceedings of the 8th International Coral Reef Symposium 2:1295-1300.

Banaszak A.T., M.P. Lesser, I.B. Kuffner, and M. Ondrusek 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. Bulletin of Marine Science 63: 617-628.

Blough N.V. and R. Del Vecchio. 2002. Chromophoric DOM in the coastal environment. *In* D.A. Hansell and C.A. Carlson (eds). Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, Amsterdam. pp. 509-546.

Carroll, A K & J.M. Shick. 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin (*Strongylocentrotus droebachiensis*). Marine Biology 124: 561-69.

Coble, P.G., R.G. Zepp, R.G. Zika (eds). 2004. Special Issue: CDOM in the ocean: Characterization, distribution and transformation. Marine Chemistry 89(1-4).

Conde, D., L. Aubriot, R. Sommaruga. 2000. Changes in UV penetration associated with marine intrusions and freshwater discharge in a shallow coastal lagoon of the southern Atlantic Ocean. Marine Ecology Progress Series 207:19-31.

Degrandpre, M.D., A. Vodacek, R.K. Nelson, E.J. Bruce and N.V. Blough. 1996. Seasonal seawater optical properties of the U.S. Middle Atlantic Bight. Journal of Geophysical Research 101:22727-22736.

D'Elia, C.F., R.W. Buddemeier and S.V. Smith. 1991. Workshop on coral bleaching. Coral Reef Ecosystem and Global Change: Report of Proceedings. College Park, University of Maryland, Maryland Sea Grant UM-SG-TS-91-03.

Dunlap W.C, and B.E. Chalker 1986. Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs 5:155-159 .

Dunlap, W.C. and J.M. Shick. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: A biochemical and environmental perspective. Journal of Phycology 34(3):418-430.

Dunne, R.P. and B.E. Brown. 2001. The influence of solar radiation on bleaching of shallow water reef corals in the Andaman Sea, 1993-1998. Coral Reefs 20:201-210.

Fitt, W.K. and M.E. Warner. 1995. Bleaching patterns of four species of Caribbean reef corals. Biological Bulletin 189:298-307.

Gibson, J. A. E., W. F. Vincent, B. Nieke, and R. Pienitz. 2000. Control of biological exposure to UV radiation in the Arctic Ocean: Comparison of the roles of ozone and riverine dissolved organic matter. Arctic 53(4):372-382.

Gleason, D.F. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreiodes*. Limnology and Oceanography 38:1452-1463.

Gleason, D.F. and G.M. Wellington 1993. Ultraviolet radiation and coral bleaching. Nature 365: 836-838.

Glynn, P.W. 1984. Widespread coral mortality and the 1982-83 El Niño warming event. Environmental Conservation 11:133-146.

Glynn, P.W. 1993. Coral reef bleaching: ecological perspectives. Coral Reefs 12:1-17.

Green, S.A and N.V. Blough. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. Limnology and Oceanography 39(8):1903-1916

Hansen, L.J. and G. DiDonato. 2002. Assessing the impacts of climate change on coral reef systems: The role of marine protected areas and terrestrial condition in adaptation strategy development. A proposal for the USEPA for cooperative agreements to provide assistance to state/tribal/other local environmental protection agencies to conduct location-specific assessment of impacts of climate change and variability on aquatic ecosystems and water quality. Ref. # CR829808010.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine Freshwater Research 50:839-866.

Hoegh Guldberg, O. and H. Hoegh-Guldberg. 2004. The implications of climate change for Australia's Great Barrier Reef. WWF Australia.

Jokiel, PL, M.P. Lesser, and M.E. Ondrusek. 1997. UV-absorbing compounds in the coral *Pocillopora damicornis*: Interactive effects of UV radiation, photosynthetically active radiation, and water flow. Limnology and Oceanography 42(6):1498-1473.

Jokiel, P.L. and S.L. Coles. 1990. Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155-162.

Kirk, J.T.O. 1994a. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press, New York. pp. 46-84.

Kirk, J.T.O. 1994b. Optics of UV-B radiation in natural waters. Ergebnisse der Limnology-Archiv fur Hydrobiologie 43:1-16.

LaJeunesse, T.C. 2001. Investigating the biodiversity, ecology and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: In search of a "species" level marker. Journal of Phycology 37:866-880.

LaJeunesse, T.C. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Marine Biology 141:387-400.

LaJeunesse, T.C., W.K.W. Loh, R. van Woesik, O. Hoegh-Guldberg, G.W. Schmidt, and W.K. Fitt. 2003. Low symbiont diversity in southern Great Barrier Reef corals relative to those of the Caribbean. Limnology and Oceanography 48: 2046-2054.

Lesser, M.P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnology and Oceanography 41:271-283.

Lesser, M.P., W.R. Stochaj, D.W. Tapley and J.M. Shick. 1990. Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation and temperature on the activities of protective enzymes against active oxygen. Coral Reefs 8:225-232.

Little A.F., M.J.H. Van Oppen, and B.L. Willis. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492-1494.

Madronich, S., R.L. McKenzie, M.M. Caldwell and L.O. Björn. 1995. Changes in ultraviolet radiation reaching the Earth's surface. Ambio 24:143-152.

Michalek-Wagner, K. 2001. Seasonal and sex-specific variation in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. Marine Biology 139:651-660.

Michalek-Wagner, K. and B. Willis. 2001. Impacts of bleaching on the soft coral *Lobophytum compactum*. II. Biochemical changes in adults and their eggs. Coral Reefs 19(3): 240-246.

Mielbrecht, E.E. and Hansen, L.J., In preparation. Are some corals better prepared for climate change: MMA concentrations in Ofu lagoon corals, Proposal. Emerald Coast Environmental Consulting, Washington, DC.

Morris, D.P. and B.R. Hargreaves. 1997. The role of photochemical degradation of dissolved organic matter in regulation of UV transparency in three lakes on the Pocono Plateau. Limnology and Oceanography 42:239-249.

Neale, P.J., A.T. Banaszak and C.R. Jarriel. 1998. Ultraviolet sunscreens *in Gymnodinium sanguineum* (dinophyceae): Mycosporine-like amino acids protect against inhibition of photosynthesis. Journal of Phycology 34: 928-938.

Pawlowski, J., M. Holzmann, J.F. Fahrni, X. Pochon, and J.J. Lee. 2001. Molecular identification of algal endosymbionts in large miliolid foraminifera: 2. Dinoflagellates. Journal of Eukaryotic Microbiology 48:368-373.

Pittock, A.B. 1999. Coral Reefs and Environmental Change: Adaptation to What? American Zoologist. 39:10-29.

Pochon, X., J. Pawlowski, L. Zaninetti, and R. Rowan. 2001. High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. Marine Biology 139:1069-1078.

Rowan, R. 1998. Diversity and ecology of zooxanthellae on coral reefs. Journal of Phycology. 34:407-417.

Rowan, R. and N. Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proceedings of the National Academy of Sciences, USA 92:2850-2853.

Rowan, R., N. Knowlton, A.C. Baker, J. Jara. 1997. Landscape ecology of algal symbiont communities explains variation in episodes of coral bleaching. Nature 388:265-269.

Rowan, R. and D. A. Powers. 1991. A Molecular Genetic Classification of Zooxanthellae and the Evolution of Animal-Algal Symbioses. Science 251:1348-1351.

Santos, S.R., C. Gutiérrez-Rodríguez, and M.A. Coffroth. 2003. Phylogenetic identification of symbiotic dinoflagellates via length heteroplasmy in domain V of chloroplast large subunit (cp23S)-ribosomal DNA sequences. Marine Biotechnology 5:130-140.

Santos, S.R., D.J. Taylor, R.A. Kinzie, M. Hidaka, K. Sakai, and M.A.Coffroth. 2002a. Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. Molecular Phylogenetics and Evolution 23:97-111.

Santos, S.R., D.J. Taylor, R.A. Kinzie, K. Sakai, and M.A. Coffroth. 2002b. Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (*Symbiodinium*, Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. Phycologia 41: 311-318.

Shick J.M., W.C. Dunlap, and G.R. Buettner. 2000. UV protection in marine organisms. II. Biosynthesis, accumulation, and sunscreening function of mycosporine-like amino acids. *In* T. Yoshikawa, S. Toyokuni, Y. Yamamoto and Y. Naito (eds). Free Radicals in Chemistry, Biology, and Medicine. OICA International, London. pp 215-228.

Shick, J.M., M. Lesser, and P. Jokiel. 1996. Effects of ultraviolet radiation on corals and other coral reef organisms. Global Change Biology 2: 527-545.

Shick, J.M., S. Romaine-Lioud, C. Ferrier-Pages and J-P. Gattuso. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. Limnology and Oceanography 44: 1667-1682.

Shick, J.M., M.P. Lesser, W.C. Dunlap, W.R. Stochaj, B.E. Chalker, and J. Wu Won. 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. Marine Biology 122:41-51.

Siegel, D.A. and A.F. Michaels. 1996. Quantification of non-algal light attenuation in the Sargasso Sea: Implications for biogeochemistry and remote sensing. Deep-Sea Research II 43:321-346.

Smith, R.C. and K.S. Baker. 1979. Penetration of UV-B and biological effective dose-rates in natural waters. Photochemistry and Photobiology 29:311-323.

Stebanau, E.R., R.G. Zepp, E. Bartels, and R.G. Zika. 2004. Role of the seagrass *Thalassia testudinum* as a source of chromophoric dissolved organic matter in coastal south Florida. Marine Ecology Progress Series 282:50-72.

Vernet M. 2000. Effects of UV radiation o the physiology and ecology of marine phytoplankton. *In* de Mora, S., S. Demers, and M. Vernet (eds). The effects of UV radiation in the marine environment. University Press, Cambridge. pp. 237-278.

Vodacek, A., N.V. Blough, M.D. DeGandpre, E.D. Peltzer, and R.K. Nelson. 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnology and Oceanography 42:674-686.

Zepp, R. 2003. UV exposure of coral assemblages in the Florida Keys. National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency Report EPA/600/R-03/095.

Table 1. Sample inventory

Sample	2002	2003			2004	
	Oct - Nov	Feb-March	May-June	Aug	Feb	June-July
Reef CDOM						
Leone	x	x	x	x	x	x
Fagatele	x	x	NA	x	x	x
Faga'alu	x	x	NA	x	x	x
Faga'itua	x	x	x	x	x	x
Vatia	x	x	x	x	x	x
Tafeu	x	NA	x	x	x	x
Malota	x	x	x	x	NA	NA
Stream CDOM						
Leone	x	x	x	x	x	x
Fagatele	NA	x	x	x	x	x
Faga'alu	x	x	x	x	x	x
Faga'itua	x	x	x	x	x	x
Vatia	x	x	x	x	x	x
Tafeu	x	NA	x	x	NA	x
Malota	x	x	x	x	x	NA
MAAs						
Fagaatele	NS	x	NA	x	x	NS
Faga'itua	NS	x	x	x	x	NS
Vatia	NS	x	x	x	x	NS
Tafeu	NS	NA	x	x	x	NS
Zooxanthellea						
Fagaatele	NS	x	NA	x	x	NS
Faga'itua	NS	x	x	x	x	NS
Vatia	NS	x	x	x	x	NS
Tafeu	NS	NA	x	x	x	NS

NA=Sampling not possible NS=No Sampling

Table 2. Mean concentrations (± SD, n=4) of mycosporine-like amino acids in coral tissues collected from sites in American Samoa from 2/2003-2/2004. Myc=Mycosporine, N.D. =Not detectable.

Collection site	Date Collected	Genus species	Concentration of MAAs (nmol/ mg protein) Myc-glycine	Myc-2-glycine	Palythine	Palythinol	Total MAAs
Fagatele	2/2003	*Acropora formosa*	62.97 ± 5.8	19.42 ± 4.1	414.56 ± 40.4	2.60 ± 2.0	499.5 ± 50.9
Fagatele	2/2003	*Acropora formosa*	143.79 ± 57.3	2.13± 0.7	495.87 ± 219.2	0	641.79 ± 276.7
Fagatele	2/2003	*Pocillopora eydoux*	139.09 ± 26.7	15.73 ± 11.3	12.33 ± 3.7	29.08 ± 15.2	196.24 ± 42.1
Faga'itua	2/2003	*Acropora formosa*	115.45 ± 22.6	2.88 ± 0.9	332.6 ± 71.0	0	450.92 ± 93.6
Faga'itua	2/2003	*Pocillopora eydoux*	48.57 ± 4.3	26.42 ± 7.3	67.34 ± 20.4	84.24 ± 10.7	226.57 ± 41.7
Vatia	2/2003	*Acropora formosa*	111.31 ± 5.9	3.44 ±3.0	453.39 ± 73.3	0	568.15 ± 78.3
Vatia	2/2003	*Pocillopora eydoux*	30.42 ± 8.3	56.64 ± 31.0	138.16 ± 73.3	73.5 ± 38.0	298.71±138.8
Tafeu	6/2003	*Pocillopora eydoux*	28.74 ± 1.7	13.53 ± 2.7	73.91 ±12.0	44.26 ± 7.5	160.44 ± 21.9
Tafeu	6/2003	*Porites rus*	69.64 ± 15,0	0.96 ± 0.3	47.48 ± 18.8	0	118.08 ± 33.8
Vatia	6/2003	*Acropora formosa*	67.41 ± 13.3	2.30 ± 0.9	210.66 ± 25.7	0	280.37 ± 39.2
Vatia	6/2003	*Pocillopora eydoux*	20.64 ± 5.4	51.69 ± 10.9	178.79 ± 23.8	91.64 ±19.3	342.76 ± 38.1
Vatia	6/2003	*Porites rus*	81.41 ± 15.8	6.77 ± 1.9	113.47 ± 12.5	0	201.65 ± 27.1
Faga'itua	6/2003	*Acropora formosa*	74.6 ± 8.4	1.75 ± 0.3	157.83 ± 32.2	0	234.22 ± 39.6
Faga'itua	6/2003	*Pocillopora eydoux*	31.54 ± 5.8	17.53 ± 2.7	64.36 ± 8.4	80.06 ± 4.5	193.48 ± 15.9
Faga'itua	8/2003	*Acropora formosa*	100.17 ± 30.2	1.77 ± 1	243.68 ± 101.6	0	345.61 ± 131.0
Faga'itua	8/2003	*Pocillopora eydoux*	27.56 ± 6.3	11.31 ± 2.6	46.95 ± 9.9	62.74 ± 15.2	148.55 ± 29.5
Fagatele	8/2003	*Acropora formosa*	78.45 ±18.4	5.01 ± 1.5	506.41 ±103.6	0.30 ± 0.6	590.17 ± 123.3
Fagatele	8/2003	*Pocillopora eydoux*	17.51 ± 2.5	20.25 ± 11.3	91.15 ± 32.7	65.77 ± 5.8	194.67 ± 50.6
Fagatele	8/2003	*Porites rus*	67.44 ± 18.4	1.84 ± 0.5	1.97 ± 1.6	25.89 ± 8.3	97.14 ± 28.1
Tafeu	8/2003	*Pocillopora eydoux*	35.76 ± 24	29.28 ± 7.3	82.79 ± 8.7	58.5 ± 6.5	206.34 ± 42.3
Tafeu	8/2003	*Porites rus*	72.15 ± 7.8	0.87 ± 0.6	1.33 ± 0.9	12.39 ±7.9	86.75 ± 10.8
Vatia	8/2003	*Acropora formosa*	63.53 ± 15.1	1.22 ± 0.5	230.72 ± 36.8	0.61 ± 0.71	296.09 ± 50.9
Vatia	8/2003	*Pocillopora eydoux*	19.76 ± 3.1	23.97 ± 8.8	81.91 ± 17.14	50.13 ± 8.8	175.77 ± 24.36
Vatia	8/2003	*Porites rus*	57.21 ± 1.3	8.33 ± 1.4	4.35 ± 0.8	37.81 ± 7.4	107.70 ± 8.9
Faga'itua	2/2004	*Acropora formosa*	73.99 ± 16.9	1.93 ± 0.4	163.9 ± 48.4	0	239.82 ± 64.3
Faga'itua	2/2004	*Pocillopora eydoux*	43.44 ± 18.4	29.04 ± 8.3	58.37 ± 12.56	69.03 ± 16.7	190.5 ± 35.6
Fagatele	2/2004	*Acropora formosa*	59.51 ± 7.4	10.68 ± 1.7	344.74 ± 37.11	2.05 ± 1.44	416.98 ± 44.1
Fagatele	2/2004	*Pocillopora eydoux*	27.71 ± 4.6	43.12 ± 10.1	127.29 ± 23.4	102.4 ± 20.8	300.54 ± 49.3
Fagatele	2/2004	*Porites rus*	65.02 ± 18.4	14.73 ± 11.5	63.39 ± 42.4	25.96 ± 34.7	169.1 ± 46.2
Tafeu	2/2004	*Pocillopora eydoux*	121.48 ± 5.7	18.54 ± 2.0	21.0 ± 1.7	17.8 ± 2.6	178.79 ± 8.5
Tafeu	2/2004	*Porites rus*	95.17 ± 14.1	2.65 ± 1.4	23.3 ± 18.6	0.77 ± 0.93	121.91 ± 33.8
Vatia	2/2004	*Acropora formosa*	43.54 ± 8.5	3.15 ± 0.6	346.55 ± 57.6	0	393.23 ± 65.3
Vatia	2/2004	*Pocillopora eydoux*	33.62 ± 8.2	68.2 ± 11.7	126.31 ± 22.9	68.8 ± 19.7	296.9 ± 51.3
Vatia	2/2004	*Porites rus*	91.14 ± 13.8	14.52 ± 3.2	135.02 ± 15.2	0.37 ± 0.73	241.05 ± 4.8

Table 3- Zooxanthellae identification for Tutuila Island, 2003 and 2004.

Sample	Location	Date	Species	Depth (feet)	*Symbiodinium* type	Preservation Technique
ASF 4	Faga'itua	3/3/03	*Acropora formosa*	15	D2	Frozen
ASF 6	Vatia	3/15/03	*Acropora formosa*	18	D2	Frozen
ASF 10	Vatia	6/11/03	*Acropora formosa*	18	D2	Frozen
ASF 13	Faga'itua	6/13/03	*Acropora formosa*	15	D2	Frozen
ASF 15	Faga'itua	8/13/03	*Acropora formosa*	15	D2	Frozen
ASF 17	Fagatele	8/13/03	*Acropora formosa*	22	C1c	Frozen
ASF 22	Vatia	8/16/03	*Acropora formosa*	18	C1c	Frozen
ASF 2	Fagatele	2/28/03	*Acropora formosa*	*No data*	D2	Frozen
AS-008	Vatia	2/24/04	*Acropora formosa*	18	D2	DMSO
AS-041	Fagatele	2/20/04	*Acropora formosa*	22	D2	DMSO
AS-043	Faga’itua	2/19/04	*Acropora formosa*	14	A3	DMSO
ASF 3	Fagatele	2/28/03	*Pocillopora eydouxi*	19	C1c	Frozen
ASF 7	Vatia	3/15/03	*Pocillopora eydouxi*	12	C3	Frozen
ASF 8	Tafeu	6/10/03	*Pocillopora eydouxi*	16	C1c	Frozen
ASF 11	Vatia	6/11/03	*Pocillopora eydouxi*	12	C3	Frozen
ASF 14	Faga'itua	6/13/03	*Pocillopora eydouxi*	20	C1c	Frozen
ASF 16	Faga'itua	8/13/03	*Pocillopora eydouxi*	20	C1c	Frozen
ASF 18	Fagatele	8/13/03	*Pocillopora eydouxi*	17	C1c	Frozen
ASF 23	Vatia	8/16/03	*Pocillopora eydouxi*	12	C1c	Frozen
AS-019	Vatia	2/24/04	*Pocillopora eydouxi*	12	C1c	DMSO
AS-035	Faga’itua	2/19/04	*Pocillopora eydouxi*	19	C1c	DMSO
AS-036	Fagatele	2/20/04	*Pocillopora eydouxi*	17	C1c	DMSO
AS-040	Tafeu	2/24/04	*Pocillopora eydouxi*	14	C1c	DMSO
ASF 9	Tafeu	6/10/03	*Porites rus*	20	C15	Frozen
ASF 12	Vatia	6/11/03	*Porites rus*	19	C15	Frozen
ASF 19	Fagatele	8/13/03	*Porites rus*	22	C15	Frozen
ASF 21	Tafeu	8/16/03	*Porites rus*	20	C15	Frozen
ASF 24	Vatia	8/16/03	*Porites rus*	19	C15	Frozen
AS-004	Vatia	2/24/04	*Porites rus*	19	C15	DMSO
AS-049	Fagatele	2/20/04	*Porites rus*	22	C15	DMSO
AS-050	Tafeu	2/24/04	*Porites rus*	20	C15	DMSO

Table 4. CDOM absorbance coefficients for reef and stream samples.

		absorbance coefficient a(λ)(m^{-1})											
		Oct-02	**Mar-03**	**Jun-03**	**Aug-03**	**Feb-04**	**Jul-04**	**Oct-02**	**Mar-03**	**Jun-03**	**Aug-03**	**Feb-04**	**Jul-04**
Sample	**Location**	**305 nm**	**305 nm**	**305 nm**	**305 nm**	**305 nm**	**305 nm**	**310 nm**	**310 nm**	**310 nm**	**310 nm**	**310 nm**	**310 nm**
Leone	Stream bottom/	1.6815	3.2585	1.9816	2.420	7.748	10.147	1.5666	3.0446	1.8417	2.254	7.294	9.507
Leone	reef	0.1821	0.1924	0.1872	0.196	0.245	0.240	0.1604	0.1709	0.1654	0.172	0.221	0.218
Fagatele	stream bottom		3.6291	3.3599	2.928	3.415	2.901		3.3471	3.0804	2.674	3.129	2.667
Fagatele	reef	0.1394	0.1548		0.200	0.267	0.281	0.1232	0.1358		0.182	0.244	0.258
Faga'alu	stream bottom	4.9101	6.2222	6.1048	2.681	8.858	3.548	4.5749	5.8225	5.7705	2.489	8.299	3.282
Faga'alu	reef	0.2670	0.1679		0.218	0.255	0.261	0.2433	0.1498		0.197	0.233	0.236
Faga'itua	stream bottom	12.6442	41.9912	8.9250	8.684	12.603	9.111	11.7691	39.4935	8.3380	8.144	11.770	8.469
Faga'itua	reef	0.1794	0.2128	0.1942	0.154	0.284	0.322	0.1621	0.1894	0.1737	0.140	0.251	0.279
Vatia	stream bottom	4.6988	9.9045	12.0611	17.152	4.846	8.370	4.4087	9.3110	11.3416	16.117	4.536	7.833
Vatia	reef	0.1617	0.2209	0.2144	0.196	0.277	0.283	0.1422	0.1956	0.1921	0.181	0.254	0.261
Tafeu	stream bottom	5.3882		3.9023	3.651		4.942	5.0502		3.6504	3.429		4.616
Tafeu	reef	0.1620		0.1733	0.207	0.254	0.284	0.1401		0.1469	0.187	0.229	0.261
Malota	stream bottom	2.7748	2.8404	2.3348	2.948	3.967		2.5987	2.6602	2.1941	2.774	3.717	
Malota	reef	0.1983	0.1816	0.2867	0.173			0.1735	0.1575	0.2614	0.157		

Table 5. CDOM spectral slope coefficients for reef and stream samples.

		Oct-02		Mar-03		Jun-03		Aug-03		Feb-04		Jul-04	
Sample	**Location**	**Spectral slope coeff.(290-400 nm)**	**r^2**	**Spectral slope coeff.(290-400 nm)**	**r^2**	**Spectral slope coeff.(290-400 nm)**	**r^2**	**Spectral slope coeff.(290-400 nm)**	**r^2**	**Spectral slope coeff.(290-500 nm)**	**r^2**	**Spectral slope coeff.(290-500 nm)**	**r^2**
Leone	Stream bottom/l	0.0154	0.9992	0.0151	0.9991	0.0156	0.9997	0.0149	0.9996	0.0135	0.9994	0.0148	0.9994
Leone	reef	0.0203	0.9900	0.0203	0.9942	0.0187	0.9794	0.0195	0.9801	0.0142	0.9774	0.0158	0.9876
Fagatele	stream bottom			0.016	0.9987	0.0181	0.9993	0.0178	0.9990	0.0181	0.9995	0.018	0.9994
Fagatele	reef	0.0203	0.9844	0.0238	0.9861			0.0174	0.9810	0.0134	0.9843	0.0146	0.9941
Faga'alu	stream bottom	0.0131	0.9994	0.0151	0.9993	0.0144	0.9894	0.0154	0.9997	0.0141	0.9997	0.0163	0.9997
Faga'alu	reef	0.0181	0.9953	0.0199	0.9867			0.0175	0.9929	0.0137	0.9863	0.0153	0.9885
Faga'itua	stream bottom	0.0149	0.9999	0.015	0.9990	0.0144	0.9995	0.0138	0.9984	0.015	0.9994	0.0159	0.9993
Faga'itua	reef	0.0172	0.9889	0.0185	0.9907	0.0190	0.9938	0.0239	0.9387	0.0159	0.9753	0.0186	0.966
Vatia	stream bottom	0.0146	0.9996	0.0143	0.9985	0.0134	0.9978	0.0142	0.9988	0.0146	0.9993	0.0144	0.9992
Vatia	reef	0.0181	0.9845	0.0185	0.9851	0.0171	0.9812	0.0179	0.9773	0.0142	0.9907	0.0152	0.9928
Tafeu	stream bottom	0.0145	0.9990			0.0141	0.9985	0.0142	0.9991			0.0147	0.9996
Tafeu	reef	0.0181	0.9671			0.0271	0.9774	0.0202	0.9822	0.0147	0.981	0.0155	0.9945
Malota	stream bottom	0.0144	0.9990	0.0145	0.9991	0.0141	0.9990	0.0147	0.9983	0.0144	0.9995		
Malota	reef	0.0171	0.9779	0.0195	0.9904	0.0160	0.9925	0.0185	0.9740				